

PATENT COOPERATION TREATY
PCT
INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)
(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference 648106C:ANB:DKM	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/AU2004/001310	International filing date (day/month/year) 24 September 2004	Priority date (day/month/year) 26 September 2003
International Patent Classification (IPC) or national classification and IPC Int. Cl. ⁷ A01H 1/06		
Applicant PHYTONOVA PTY LTD et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 4 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
 - a. ☒ (sent to the applicant and to the International Bureau) a total of 9 sheets, as follows:
 - ☒ sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
 - ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
 - b. ☐ (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:

<input checked="" type="checkbox"/> Box No. I	Basis of the report
<input type="checkbox"/> Box No. II	Priority
<input type="checkbox"/> Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
<input type="checkbox"/> Box No. IV	Lack of unity of invention
<input checked="" type="checkbox"/> Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
<input type="checkbox"/> Box No. VI	Certain documents cited
<input checked="" type="checkbox"/> Box No. VII	Certain defects in the international application
<input type="checkbox"/> Box No. VIII	Certain observations on the international application

Date of submission of the demand 26 July 2005	Date of completion of the report 4 August 2005
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer LEXIE PRESS Telephone No. (02) 6283 2677

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

PCT/AU2004/001310

Box No. I **Basis of the report**

1. With regard to the language, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
- ☐ This report is based on translations from the original language into the following language which is the language of a translation furnished for the purposes of:
- ☐ international search (under Rules 12.3 and 23.1 (b))
 - ☐ publication of the international application (under Rule 12.4)
 - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the elements of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):
- ☐ the international application as originally filed/furnished
- ☒ the description:
- pages 4-12 as originally filed/furnished
- pages* 1, 2, 3, 3a received by this Authority on 26 July 2005 with the letter of 26 July 2005
- pages* received by this Authority on with the letter of
- ☒ the claims:
- pages as originally filed/furnished
- pages* as amended (together with any statement) under Article 19
- pages* 13-17 received by this Authority on 26 July 2005 with the letter of 26 July 2005
- pages* received by this Authority on with the letter of
- ☐ the drawings:
- pages as originally filed/furnished
- pages* received by this Authority on with the letter of
- pages* received by this Authority on with the letter of
- ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.
3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
 - ☐ any table(s) related to the sequence listing (*specify*):
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
 - ☐ any table(s) related to the sequence listing (*specify*):

* If item 4 applies, some or all of those sheets may be marked "superseded."

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

PCT/AU2004/001310

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-48	YES
	Claims	NO
Inventive step (IS)	Claims 1-48	YES
	Claims	NO
Industrial applicability (IA)	Claims 1-48	YES
	Claims	NO

2. Citations and explanations (Rule 70.7)

In general, the application is directed to methods for inducing polyploidy in woody perennials by the application of colchicine to buds. In a preferred embodiment buds are treated with 0.5%w/v to 3% w/v colchicine and exposed to substantially constant illumination until growth from the treated tissue occurs.

The amendments and submissions of 26 July 2005 and the documents cited in the ISR have been considered for the basis of this report:

D1 Lapins K. O. (1975) Can. J. Genet. Cytol. Vol 17: 591-599

D2 Ivanička et al (1988) Acta Hort. Vol 224: 429-435

D3 Barrett H.C. (1974) Bot Gaz. Vol 135(1): 29-41

D4 Bringhurst R. S. (1956) Proceedings of the American Society for Horticultural Science. Vol 67: 251-257

D5 Liu et al (2001) Genome. Vol 44: 321-330

D6 Rey et al (2002) Euphytica. Vol 123: 49-56

D7 Iwanaga et al (1991) Genome. Vol 34: 201-208

D8 Zeldin E. L. & B. H. McCown (2002) Acta. Hort. Vol 574: 175-180

D9 McCuiston F. & T.C. Wehner (2002) <http://www.cuke.hort.ncsu.edu/cucurbit/wmelon/seedless.html>

Novelty and Inventive Step

The prior art is directed to colchicine induced polyploidy in plants and the use of polyploids in plant breeding programs. However, there is no disclosure or suggestion in the prior art of the specific methods as taught in the present application. Claims 1-48 meet criteria set forth in PCT Article 33(2) for novelty, and PCT Article 33(3) for inventive step.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

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Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

As a result of amendments there are two pages numbered 17.

Method for increasing ploidy in a plant

Technical Field

The present invention relates generally to the field of plant breeding and the production of plants and plant breeding lines comprising improved or new desirable traits. In particular, the invention relates to methods of increasing the ploidy of a plant species or plant variety. The invention also relates to plant varieties and species produced thereby and to methods of generating new plant varieties comprising at least one desirable trait.

Background of the Invention

The manipulation of ploidy in plant tissue has been used, for example, to produce fruit without seeds, to introduce fertility into hybrids that otherwise would not normally be able to be created, and to produce plants with other desirable characteristics, such as, in flowering plants, larger blooms or more intense colour.

Plant breeders use a naturally occurring compound, colchicine, which inhibits the development of spindle fibres during nuclear or cell division, to increase the chromosome number (for example, doubling up the DNA of a species). Then, with a matched chromosome number, new hybrids can be produced that could not otherwise be created.

Current methods used for increasing chromosome number in plants generally employ colchicine concentrations in the order of 0.001% w/v to 0.2% w/v. The percentage of cells of the exposed plant tissue in which chromosome multiplication has been effected by known methods is generally very low. Furthermore, the reliability of the known methods, in terms of reproducibly attaining even low percentages of cells in which ploidy has increased after exposure is variable. As a result, production of, for example, new breeding lines or plants having desirable traits by the known methods is inefficient.

Summary of the Invention

It has surprisingly been found that exposure of plant tissue to relatively high concentrations of colchicine results in improved yield of cells in which chromosome multiplication has been effected. It has also surprisingly been found that improved yields may be attained more reproducibly than is attained with known methods. In addition, the yield of cells having an increase in ploidy may be improved when exposure of the plant tissue to the agent capable of inhibiting spindle formation is commenced substantially coincidental with the breaking of dormancy of the plant tissue. That is, when the plant tissue or plant is put in an active state with respect to cell division.

Accordingly, herein disclosed is a method of increasing ploidy in cells of a woody perennial plant, the method comprising:

contacting plant tissue comprising dividing cells with an effective amount of a composition comprising an agent capable of inhibiting spindle formation, wherein said

contacting commences substantially coincidental with breaking dormancy of said plant tissue.

According to an aspect of the invention, there is provided a method of increasing ploidy in cells of a woody perennial plant, the method comprising:

5 contacting a bud of said plant substantially continuously over a period of at least 1 day with an effective amount of a composition comprising an agent capable of inhibiting spindle formation, wherein said contacting commences substantially coincidental with breaking dormancy of said plant tissue, wherein said bud comprises actively dividing cells and is apically or terminally dominant; and

10 exposing the treated bud to substantially constant illumination until growth from the treated tissue occurs.

According to an embodiment of the methods of the invention, the plant tissue may be at least one bud grafted onto a rootstock plant. The apical shoot and all buds of the rootstock plant may be removed, so as to allocate more of the plant resources to the grafted bud(s). The plant tissue may be a single grafted bud.

Thus, according to another aspect of the invention, there is provided a method of increasing ploidy in cells of a woody perennial plant, the method comprising:

15 contacting a bud of said plant substantially continuously over a period of at least 1 day with an effective amount of a composition comprising an agent capable of inhibiting spindle formation, wherein said contacting commences substantially coincidental with breaking dormancy of said plant tissue, wherein said bud is grafted onto a rootstock plant, comprises actively dividing cells and is apically or terminally dominant.

The plant tissue may be exposed to ultraviolet, or fluorescent light or to a mercury and/or sodium lamp substantially continuously subsequent to said contacting at least until growth from the treated tissue occurs.

25 Also herein disclosed is a method of increasing ploidy in cells of a woody perennial plant, the method comprising:

contacting plant tissue comprising dividing cells with an effective amount of a composition comprising about 0.5% w/v colchicine to about 3% w/v colchicine.

30 According to another aspect of the invention, there is provided a method of increasing ploidy in cells of a woody perennial plant, the method comprising:

35 contacting a bud of said plant substantially continuously over a period of at least 1 day with an effective amount of a composition comprising from about 0.5% w/v colchicine to about 3% w/v colchicine, wherein said bud comprises actively dividing cells and is apically or terminally dominant; and

exposing the treated bud to substantially constant illumination until growth from the treated tissue occurs.

According to another aspect of the invention, there is provided a method of increasing ploidy in cells of a woody perennial plant, the method comprising:

contacting a bud of said plant substantially continuously over a period of at least 1 day with an effective amount of a composition comprising from about 0.5% w/v colchicine to about 3% w/v colchicine, wherein said bud has been grafted onto a rootstock plant, comprises actively dividing cells and is apically or terminally dominant.

5 Also herein disclosed is a method of increasing ploidy in cells of a deciduous woody perennial plant, the method comprising:

contacting at least one bud of said plant, wherein said bud comprises actively dividing cells, with a composition comprising about 0.5% w/v colchicine to about 3% w/v colchicine,

10 at least partially enveloping said bud with a material capable of inhibiting gaseous exchange, wherein said contacting is substantially continuous over a period of from about 5 days to about 15 days.

According to another aspect of the invention, there is provided a method of increasing ploidy in cells of a woody perennial plant, the method comprising:

15 contacting at least one apically or terminally dominant bud of said plant, wherein said bud comprises actively dividing cells, with a composition comprising about 0.5% w/v colchicine to about 3% w/v colchicine,

at least partially enveloping said bud with a material capable of inhibiting gaseous exchange, wherein said contacting is substantially continuous over a period of from about 20 5 days to about 15 days; and

exposing the treated bud to substantially constant illumination until growth from the treated tissue occurs.

According to another aspect of the invention, there is provided a method of increasing ploidy in cells of a woody perennial plant, the method comprising:

25 contacting at least one bud of said plant with a composition comprising about 0.5% w/v colchicine to about 3% w/v colchicine, wherein said bud has been grafted onto a rootstock plant, is apically or terminally dominant and comprises actively dividing cells; and

30 at least partially enveloping said bud with a material capable of inhibiting gaseous exchange, wherein said contacting is substantially continuous over a period of from about 5 days to about 15 days.

Also herein disclosed is a method of generating a plant having a desired ploidy level, the method comprising:

35 contacting plant tissue comprising dividing cells with an effective amount of a composition comprising about 0.5% w/v colchicine to about 3% w/v colchicine,

generating at least one plant from tissue so contacted, and
selecting at least one plant having the desired ploidy level.

Also herein disclosed is a method of generating a plant, the method comprising:

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contacting plant tissue comprising dividing cells with an effective amount of a composition comprising about 0.5% w/v colchicine to about 3% w/v colchicine,

selecting plant tissue of increased ploidy level,

generating at least one plant from said selected plant tissue, and

5 crossing said generated plant with a plant of the same or different ploidy level.

Also herein disclosed is a method of generating a plant having at least one desired trait, the method comprising:

contacting plant tissue comprising dividing cells with an effective amount of a composition comprising about 0.5% w/v colchicine to about 3% w/v colchicine,

10 selecting plant tissue of increased ploidy level,

generating at least one plant from said selected plant tissue,

crossing said generated plant with a plant of the same or different ploidy level, and

selecting at least one progeny plant having the desired trait.

Also herein disclosed is a method of generating a plant having at least one desired trait, the method comprising:

contacting parental diploid plant tissue comprising dividing cells with an effective amount of a composition comprising about 0.5% w/v colchicine to about 3% w/v colchicine,

20 selecting tetraploid tissue from said treated plant tissue,

generating at least one tetraploid plant from said tetraploid tissue,

crossing said tetraploid plant with a diploid plant, and

selecting at least one progeny plant having the desired trait.

In a further aspect, the invention relates to a plant or propagative material thereof, of fruit thereof, produced by a method of the invention.

25

Detailed Description and Examples

It has surprisingly been found that the use of hitherto believed excessive concentrations of an agent capable of inhibiting spindle formation is advantageous in inducing increased ploidy in plant tissue. The agent capable of inhibiting spindle formation in the plant tissue may be any suitable agent, for example colchicine, oryzalin (SurflanTM), trifluralin, amiprofos-methyl, and N₂O gas. It is also envisaged that a combination of agents may be used.

Where the agent capable of inhibiting spindle formation is colchicine, the colchicine may be administered as a composition comprising about 0.5% w/v colchicine to about 3% w/v colchicine. Thus, the composition may comprise colchicine in a concentration of about 0.5%w/v, about 0.6% w/v, about 0.7% w/v, about 0.8% w/v, about 0.9% w/v, about 1%w/v, about 1.1% w/v, about 1.2% w/v, about 1.3%w/v, about 1.4% w/v, about 1.5% w/v, about 1.6% w/v, about 1.7% w/v, about 1.8% w/v, about 1.9% w/v, about 2% w/v, about 2.1% w/v, about 2.2% w/v, about 2.3% w/v, about 2.4% w/v, about 2.5% w/v, about 2.6% w/v, about 2.7% w/v, about 2.8% w/v, about 2.9% w/v or about 3% w/v.

Claims

1. A method of increasing ploidy in cells of a woody perennial plant, the method comprising:

5 contacting a bud of said plant substantially continuously over a period of at least 1 day with an effective amount of a composition comprising from about 0.5% w/v colchicine to about 3% w/v colchicine, wherein said bud comprises actively dividing cells and is apically or terminally dominant; and

exposing the treated bud to substantially constant illumination until growth from the treated tissue occurs.

10 2. The method of claim 1, wherein said bud is a bud that has been grafted onto a rootstock plant.

3. A method of increasing ploidy in cells of a woody perennial plant, the method comprising:

15 contacting a bud of said plant substantially continuously over a period of at least 1 day with an effective amount of a composition comprising from about 0.5% w/v colchicine to about 3% w/v colchicine, wherein said bud has been grafted onto a rootstock plant, comprises actively dividing cells and is apically or terminally dominant.

4. The method of claim 3, further comprising the step of exposing the treated bud to substantially constant illumination until growth from the treated tissue occurs.

20 5. The method of claim 2 or claim 3 wherein the apical shoot and all buds of the rootstock plant have been removed.

6. The method of claim 1 or claim 3, wherein said bud is the sole bud on said plant.

25 7. The method of claim 1 or claim 3 wherein the concentration of colchicine is about 0.8% w/v to about 1.5% w/v.

8. The method of claim 1 or claim 3 wherein the concentration of colchicine is about 1% w/v.

9. The method of claim 1 or claim 3 wherein the woody perennial plant is a deciduous woody perennial.

30 10. The method of claim 1 or claim 3 wherein the plant is selected from the group consisting of a *Prunus* spp, *Pyrus* spp, *Malus* spp, *Citrus* spp, *Poncirus* spp, *Persea* spp, *Mangifera* spp, *Punica* spp, and *Olea* spp.

35 11. The method of claim 1 or claim 3 wherein the method further comprises prior to said contacting step exposing said plant tissue to conditions sufficient to break dormancy of said bud.

12. The method of claim 11 wherein conditions sufficient to break dormancy of said bud comprise maintaining said bud at an appropriate temperature for a time sufficient to satisfy the chill requirement of said bud, optionally in the presence of hydrogen cyanimide, and maintaining said bud at an appropriate temperature for a time
5 sufficient to prime cell division in said bud.

13. The method of claim 1 or claim 3 wherein said contacting comprises at least partially enveloping said bud with an absorbent material.

14. The method of claim 13 wherein said absorbent material is a cotton based material, or sponge or sponge-like material or foam.

10 15. The method of claim 14 wherein said cotton based material is cotton wool.

16. The method of claim 1 or claim 3 wherein said bud, and surrounding tissue, is at least partially enveloped with a material capable of inhibiting gaseous exchange.

15 17. The method of claim 16 wherein said material capable of inhibiting gaseous exchange is a plastic film.

18. The method of claim 1 or claim 3 wherein the composition further comprises one or more agents or carriers capable of enhancing plant tissue penetration of said colchicine.

20 19. The method of claim 18 wherein the agent capable of enhancing plant tissue penetration is selected from the group consisting of surfactants, wetting agents, oils and dimethylsulfoxide.

20. The method of claim 1 or claim 3 wherein said contacting comprises substantially continuous exposure of said tissue to said composition over a period from about one day to about 30 days.

25 21. The method of claim 1 or claim 3 wherein said contacting comprises substantially continuous exposure of said tissue to said composition over a period from about 5 days to about 15 days.

30 22. The method of claim 1 or claim 3 wherein said contacting comprises substantially continuous exposure of said tissue to said composition over a period of about 10 days.

23. The method of claim 1 or claim 3 wherein said contacting comprises multiple applications of said composition.

24. The method of claim 23 wherein said multiple applications comprise two or more applications per day.

25. The method of claim 23 wherein at least one of said applications is administered when plant cell division is substantially maximal.

26. The method of claim 1 or claim 3 wherein said bud is exposed to ultraviolet, or fluorescent light or a mercury and/or sodium lamp.

5 27. The method of claim 26, wherein said plant tissue is exposed to ultraviolet, or fluorescent light or to a mercury and/or sodium lamp prior to said contacting as well as substantially continuously subsequent to said contacting at least until growth from the treated tissue occurs.

10 28. The method of claim 1 or claim 3, wherein plant tissue arising from the treated bud, or cells at the base of the bud are checked for ploidy and tissue having the desired ploidy level selected.

29. The method of claim 28, wherein the plant or plants grown from the selected tissue is/are tested for inheritability of the desired ploidy level.

15 30. A method of increasing ploidy in cells of a woody perennial plant, the method comprising:

contacting at least one apically or terminally dominant bud of said plant, wherein said bud comprises actively dividing cells, with a composition comprising about 0.5% w/v colchicine to about 3% w/v colchicine,

20 at least partially enveloping said bud with a material capable of inhibiting gaseous exchange, wherein said contacting is substantially continuous over a period of from about 5 days to about 15 days; and

exposing the treated bud to substantially constant illumination until growth from the treated tissue occurs.

25 31. The method of claim 30, wherein said bud has been grafted onto a rootstock plant.

32. A method of increasing ploidy in cells of a woody perennial plant, the method comprising:

30 contacting at least one bud of said plant with a composition comprising about 0.5% w/v colchicine to about 3% w/v colchicine, wherein said bud has been grafted onto a rootstock plant, is apically or terminally dominant and comprises actively dividing cells; and

at least partially enveloping said bud with a material capable of inhibiting gaseous exchange, wherein said contacting is substantially continuous over a period of from about 5 days to about 15 days.

33. The method of claim 32, further comprising the step of exposing the treated bud to substantially constant illumination until growth from the treated tissue occurs.

34. The method of claim 31 or claim 32, wherein said bud is the sole bud on said rootstock plant.

35. The method of claim 30 or claim 32 wherein the concentration of colchicine is about 0.8% w/v to about 1.5% w/v.

36. The method of claim 30 or claim 32 wherein the concentration of colchicine is about 1% w/v.

37. The method of claim 30 or claim 32 wherein the bud is from a deciduous woody perennial plant.

38. The method of claim 30 or claim 32 wherein the plant is selected from the group consisting of *Prunus* spp, *Pyrus* spp, and *Malus* spp.

39. The method of claim 30 or claim 32 wherein the method further comprises prior to said contacting step exposing said plant tissue to conditions sufficient to break dormancy of said bud.

40. The method of claim 30 or claim 32, further comprising the steps of:
generating at least one plant from a bud so contacted; and
selecting at least one plant having a desired ploidy level.

41. The method according to claim 40 wherein the desired ploidy level is diploid (2N), tetraploid (4N) or hexaploid (6N), octoploid (8N), decaploid (10N) or dodecaploid (12N).

42. A method of generating a woody perennial plant having at least one desired trait, the method comprising:

generating tetraploid plant tissue from a bud of a diploid parental woody perennial plant variety by a method of any one of claims 1, 3, 30 or 32;
generating at least one tetraploid plant from said tetraploid tissue;
crossing said tetraploid plant with a diploid plant;
generating at least one progeny plant having the desired trait.

43. The method of claim 42 wherein the desired trait is seedlessness.

44. The method of claim 42 wherein said tetraploid plant is back-crossed with said diploid parental woody perennial plant variety.

45. The method of any one of claims 1 to 44 wherein said contacting commences substantially coincidental with breaking dormancy of said bud.

46. A method of increasing ploidy in cells of a woody perennial plant, the method comprising:

contacting a bud of said plant substantially continuously over a period of at least 1 day with an effective amount of a composition comprising an agent capable of inhibiting spindle formation, wherein said contacting commences substantially coincidental with breaking dormancy of said plant tissue, wherein said bud comprises actively dividing cells and is apically or terminally dominant; and

exposing the treated bud to substantially constant illumination until growth from the treated tissue occurs.

47. A method of increasing ploidy in cells of a woody perennial plant, the method comprising:

contacting a bud of said plant substantially continuously over a period of at least 1 day with an effective amount of a composition comprising an agent capable of inhibiting spindle formation, wherein said contacting commences substantially coincidental with breaking dormancy of said plant tissue, wherein said bud is grafted onto a rootstock plant, comprises actively dividing cells and is apically or terminally dominant.

48. The method of claim 47, further comprising the step of exposing the treated bud to substantially constant illumination until growth from the treated tissue occurs.